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Physico-chemical Properties of Nutmeg (*Myristica fragrans houtt*) of North Sulawesi Nutmeg

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ABSTRACT

Essential oil of nutmeg (*Myristica fragrans houtt*) is one of the many potentials of nutmeg that has a high economic value although in North Sulawesi it has not been exploited to its full potential. This research was conducted to compare the yield and properties of Nutmeg oil extract from the seeds and mace of Talaud and North Minahasa -North Sulawesi. The oil extract was obtained by distillation and was further characterized in terms of color, solubility in ethanol, density, optical rotation, refractive index. Results of this research exhibited that oil of the mace of nutmeg from Talaud had a lighter appearance in color compared to that from North Minahasa, while the density of oil extracted from North Minahasa was more densed compared to Talaud in both seed and mace (0.923 and 0.938 respectively at 25°C). The reflective index of nutmeg oil from North Minahasa was slightly higher than of Talaud both from seed and mace (1.4834 and 1.493 at 25°C), while the optical rotations of oil extracted from the mace were between +6.90° to +9.80° and from the seed were +20.73° to +22.30°.9.

Introduction

Rismunandar, described the *Myristica fragrance* houtt, the Indonesian Nutmeg originated from the island of Banda [1]. The nutmeg tree grows well in the tropical climate, and found on the continents of the America, Asia and Africa. Worldwide, there are 250 species of nutmeg, but only a hand fold has economical value including the *Myristica fragrans HOUTT* (Banda nutmeg), *Myristica specisa WARB* (Bacan Nutmeg), *Myristica argenia WARB* (Papua Nutmeg), *Myristica succedena BLUME* (Halmahera Nutmeg), and the *Myristica malabarica* (Malabar Nutmeg). Along the Indonesian archipelago, areas of nutmeg producers are Maluku, North Sulawesi, West Sumatera, Nanggroe Aceh Darussalam, West Jawa and Papua [2].

The mature nutmeg fruit appears yellowish green with a tough texture and whitish flesh. 3-9 cm in diameter, and it can be harvested at 6-9 months after flowering. The fruit itself would consist of the flesh (77,8%) and seed (13,1%) which would consist of the mace (4,0%), and the shell (5,1%). The shell of the seeds is tough; mainly dark chocolate in color. Between the flesh and the seed would appears a layer of reddish aril that would envelope the seed with a distinct nutmeg aroma and flavor although more delicate [2, 3].

Phytochemical analysis of nutmeg contains essential oils, protein, lipids, starches and various residues. The essential oil of were mainly myristicin and myristic acid, while the oil alone contains approximately 5-15% of pine, sabinene, camphene, myristicin, elemicin, isoelemicin, eugenol, isoeugenol, methoxyeugenol, safrole, diametricphenylpropanoids, lignans and neolignans [4, 5].

In several European countries, nutmeg in small amounts is mainly used as spices and ingredient in the culinary aspects mainly to support meaty flavors or in soups. Nutmeg oil as a flavor enhancer should not be more than 0.08%, beyond that may induce intoxication. Nutmeg oil is also known for other properties, such as insecticide, fungicide, and antibacterial. A study on the anti-oxidative activities of nutmeg seeds in comparison with BHT, ascorbic acid and α-tocopherol. Results of the work of indicated that nutmeg essential oil had a very strong anti-oxidative characteristic, which was due to synergism of each components of the essential oil [6].

Nutmeg oil in the pharmaceutical industry has been utilized as abdominal medications, diarrhea and bronchitis; moreover, nutmeg may be useful for treating flatulence, increasing metabolism, treating dysentery (infectious diarrhea), stomach inflammation, a remedy of vomiting, constipation, bloating and rheumatism. 2-18% of the aromatic compounds of myristicin, elimicin, and safrole contained in the seed and flowers has the ability to induced hallucination [7]. Consumption of up to 5 grams of nutmeg powder or oil may cause intoxication diagnosed by vomiting, headaches and dry mouth [8-10]

Nutmeg is also a known aphrodisiac, stomachic, carminative while Guenther, (1952), also noted that nutmeg has the function as a stimulant (tonic), nervous stimulant, aromatic, narcotic, astringent, holypolipidemic, anti-thrombotic, anti-fungi, anti-dysentry and anti-inflammation [11, 12]. Nutmegs are also reported to be very useful in treating paralysis and help to improve blood circulation [13]. The Nutmeg also exhibited anti-oxidative characters [6, 14].

Petroleum ether extract of the flesh of *Myristica fragans* exhibited anti-diarrhea, while extracts by n-hexane has been reported to have the capacity to improve memory in rats [15]. Nutmeg appears to have anti-bacterial activities towards *Staphylococcus aureus*, *Proteus vulgaris* and *Klebsiella pneumonia*, but was not active towards *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Bacillus subtilis [6]*.

The nutmeg oil appears clear and a little yellowish, faintly green at certain species and has a mild delicate aroma specific to nutmeg. Nutmeg oil is very sensitive towards light and temperature, very soluble in ethanol but not water soluble [12]. Asside of oil, nutmeg contains a buttery lipid regarded as oleoresin or nutmeg butter. The main constituent of this butter are oleoresin, and especially trimyristicin [12].

Nutmeg oil composition consists of 61-88% hydrocarbons (monoterpen), 5-15% oxygenated hydrocarbon and 2-18% aromatic ether, and other smaller compounds in minute compositions [3]. The largest constituent of monoterpen hydrocarbons are the α-pinene (17,2%), β-pinene (23,9%) and sabinene, meanwhile the myristicin are also considered as one of the main components especially from the aromatic esther fractions, this includes miristic acid that was also found in smaller ammounts. The distinct fragrant aroma of the oil is mainly caused by the aromatic compound, in this case are the myristicin (16,2%), safrole (3,9%) and methyl eugenol (1,8%), furthermore there are 25 identified components of nutmeg oil (92,1% of the total oil), that was all obtained by hydrodestillation.

One of the quality parameters of industrially collected nutmeg oil is determined by the myristin content, since myristicin contributes to specific fragrant aroma of the nutmeg [9]. Myristicins are derivatives of the phenilepropanoid compounds, a clear to opaque liquid that is not soluble in water but organic solvents, it has a distinct sharp herbal aroma, and is very volatile. With a molecular weight of 192 g/mol, the other name of myristicin is 5-alil-1-methoxi-2,3 methilene dioxibenzene or 5-metoxi saphrole [9].

The nutmeg oil is commonly obtained by the simple steam destillation method or hydrodestillation. Extractions by those methods will yield about 5% to 15% [16]. Destillation of the nutmeg oil is commonly from the seeds and mace of the nutmeg. The seeds that are mostly used for oil extraction are usually the younger seeds due to the fact that the oil content would be higher than the older seeds, where the oil would be more pale yellow in color, very volatile and extends a very distinct fragrant note [2].

Aside of the distillation methods

commonly used such as steam/water distillation or a combination of steam and water distillation method, it is also common to find a method of distillation also with water known as the *cohobation* method, unfortunately this method is less popular due to the quality of the oil obtained varies below the standards [3].

Yield and quality of the distillation results depends on factors of source material to be extracted, method and conditions of the distillation, the installation of the distillatory, continue that nutmeg oil in Indonesia are most commonly originated from the seeds aging about 3-4months with a good yield of 8-17% but at a very low quality, while the older seeds has a lower yield of 8-13% but a higher quality of oil [17].

Nutmeg oil can also extracted from the mace and leaves, while from the mace it is still achievable to extract between 4-17% but from the leaves only could only yield up to 1.7%, thus trying to extract oil from the leaves would be very uneconomical, even though chemically the characteristics of the oil from the seed, mace even the leaves were similar quality-wise the leaves were less [18, 19]. Scientifically, advancements in the extraction of nutmeg oil components have moved from simple steam and water to the utilization of super critical CO2, or the supercritical fluid with a cellulose acetate reverse osmosis membrane [17, 20].

Materials and Methods

Materials

The main material of this research is the nutmeg itself which consists of the mace, and seed. Samples were obtained at Talaud Regency and North Minahasa Regency, North Sulawesi.

Sample Preparation

The nutmeg samples obtained from both regencies were cut up in pieces and separated between the mace and seeds. The nutmeg samples used in this research were fruits that are young but physiologically mature at about 5-6 months after the bloom.

The sample cuts were then spread evenly on a flat bamboo tray where the base section was partially perforated due to the spaces between the bamboo weavings. This is necessary that the samples would receive ample aeration during the drying. Drying the sample wasn't executed under any source of direct heat source, not even direct sunlight, only by placing the trays of samples in a cool dry area with ample wind.

Destillation Nutmeg Oil

The destillation of the nutmeg oil was conducted with a simple steam destillation method as described by Adewole and Marzuki with modification to adapt the apparatus to develop a simple apparatus that can be made industrial at the farmer level [14, 21].

Physcochemical Analysis

The nutmeg essential oil physicochemical analysis begins from the oil yield, visual appearances, and solubility in alcohol, density, optical rotation and refraction indexes; where all were done accordingly to the SNI (Standard Nasional Indonesia).

Color analysis of the oil was done empirically, by observing the physical appearances of the oil obtained by distillation. The yield percentage of the oil was calculated as the proportion of nutmeg oil in sample volume, as the formula as follow:

$$\text{Nutmeg yield} = \frac{Nutmeg\ oil\ volume}{sample\ volume} \times 100\%$$

Solubility in alcohol was conducted according the method of guenther, where 1 ml essential oil was added ethanol a drop at a time [12]. With each addition of ethanol drop it the mixture was shaken and observed if the oil is soluble or not. The addition of ethanol continues until a clear solution is obtained.

Density was done with a method as carried out by Guenther which was simply using a picnometer [12]. The Picnometer was washed and cleaned with ethanol and afterwards flushed with ether. Once dried it was weighed on a digital scale, following the picnometer was then filled with ddH_2O until the given mark and closed. The Picnometer filled with distilled water was left for a while before it was weighed once more. Water equivalent picnometer was the weight of the picnometer filled substracted with the empty weight. This method was then conducted with the nutmeg oil and the density was calculated with the following formula:

$$\text{Density } (t^0C) = \frac{\text{density of numeg oil}}{\text{density of } ddH_2O}$$

$$\text{Density } (25^0C) = d + 0.00082\ (t\text{-}25^0C)$$

where:

t = temperature
d= density of nutmeg oil at temperature (t^0C)
0.00082 = nutmeg oil correction factor

Optical rotation was conducted with the polarimeter also following what was done by Guenther (1952) [12]. This was done by filling the polarimeter tube with nutmeg oil sample and then placed under the apparatus to be checked between polarity and analizer. The analizer was then turned slowly until half is seen through the telescope, and the light intensity appears as bright as the light source. At the proper adjustments accordingly to the right or left rotation based on the rightness on the fields. Determination of the direction of rotation were as to where the analizer was rotating; counter-clockwise from zero was named levo (-), while clockwise dextro (+). After the determination of the direction of rotation the analizer was rearranged until the light intensity was again equal for the both areas. Observation was conducted through the scopes while rotating the analizer, then the lines between the two fields becomes clear and thus the value was read. The blanks were also conducted through the same procedure.

Optical Rotation = sample readout – blanks readout

The refractive indexes were carried out using a refractometer. The nutmeg oil sample was placed on the prism of the refractometer at about 2-3 drops to cover the surface of the prisms; the prisms were then closed together and left aside for a while for the oil temperature to adjust evenly. The slides where then arranged to observe a clear line between the light and the dark and when the lines meet and crosses is the refraction indexes are read. The refraction indexes will then be formulized as follow:

$$n^tD = n^{t1}D + 0.00046\ (t_1\text{-}t)$$

where:

n^tD = oil refraction indexes at a certain temperature and density
$n^{t1}D$ = oil refraction indexes at temperature ($t_1{}^0C$) and density D
t = comparison temperature
D = oil density
t_1 = temperature test conducted on
0.00046 = refraction correction factor at each 1^0C

Results and Discussion

The nutmeg oil that was obtained yield as distillation results were 2.41% and 3.66% respectively for the seeds and the mace. These yields were confirmed a work on the dry seeds of the North Sulawesi Islands (Sangihe, Talaud and Siau) and the North Sulawesi mainland (Minahasa Utara, Minahasa Tenggara and Minahasa Induk) where the results not far off with what was obtained in this research where the seeds of the off shore islands of North Sulawesi mainly 4.89-5.11% were about while the on the mainland are about 4.08-5.01% [22]. The highest yield destillated from the islands of Siau were 5.11% and the lowest were from Southwest Minahasa (4.08%). Other researches that was conducted in Papua exhibitited a lower yield of 3.11%, while of the seeds of Banda island was in the range of 8-12% [23]. Kartini also collected a yield of 2.25-3.35% from Papua nutmegs [24].

The color of nutmeg maces of Talaud were yellowish to brownish in color, the North Minahasa maces were more brownish in color, while nutmeg oil obtained from the seeds from Talaud or North Minahasa are more yellowish (Fig 1.). The color of the nutmeg oil of seeds and maces produced all were basically pale yellow, and was within the standard of SNI No. 06-2388-2006 (Indonesian National Standard) that has set the standards that the color of nutmeg oil was pale yellow to clear, with a distinct fragrant note of the nutmeg.

Figure 1. Collected Appearances of The Nutmeg Oil

Darker in colors according to [2], indicates the presences of more pigments and residual components that was collected along with the oil during the distillation process, which in this case the yellowish color would more likely be flavonoids and the more darker brownish-reddish colors would more likely be due to the presences of terpenoids. The clear to yellowish color of the nutmeg oil also may be an indication that the oils are still in a good condition, uninfluenced by air or sunlight, while a tendency for a darker color maybe an indication that the oil has undergone oxidation and has absorbed oxygen from the air into the oil. Beyond color, the oxidation process also will eventually have an impact on the aroma, the process will transform the fragrant mild sweet aroma to a thicker heavier aroma while the oil itself will also thickens and finally form resins [25]. Idrus *et al*. (2014) mentioned in their work that the aroma of nutmeg was mainly due to the presences of compounds in the oil in which ± 88% of it consists of monoterpene hydrocarbons, with the main components of camphene, pinene, myristicin, and monoterpen alcohols like: geraniol, lonalool, terpineol, eugenol and methyl eugenol [26].

In regards with the density of the nutmeg oils, Tabel 1 exhibited densities of the oil extracted from the nutmeg's mace originating from Talaud and North Minahasa that was measured at 25^{0}C were 0.9459 and 0.9538 (respectively), while the density for the oils from the seeds of Talaud and North Minahasa were 0.9099 and 0.9230. In comparison with previous research on nutmeg off the of shore islands and mainland North Sulawesi (Table 2), on Banda Nutmeg, on Papua Nutmeg, and according Standar Nasional Indonesia (Tabel 3) all appeared that the density of oil from the various parts of the nutmeg fruit are greater than the standard that was by SNI (0,880-0,910) [22, 26]. Density of nutmeg oil Talaud and North Minahasa was similar the density of nutmegs from Banda and Papua, while the density of nutmeg of offshore and mainland of North Sulawesi are between 0.860–0.883 which are mainly from Sangihe and Siau.

Table 1. Characteristics and Myristicin Content of Nutmegs of North Minahasa and Talaud

Parameter	Mainland (North Minahasa)		Offshore Islands (Talaud)	
	Seeds	Mace	Seeds	Mace
Density	0.9230	0.9538	0.9099	0.9459
Optical Rotation (25°C)	+20.73°	+6.90°	+22.30°	+9.80°
Refractive Indexes (25°C)	1.4834	1.4934	1.4807	1.4909
Solubility in Alcohol	1:1 (Soluble)	1:1 (Soluble)	1:1 (Soluble)	1:1 (Soluble)
Myristicin Content	13.29%	18.80%	10.93%	18.97%

Table 2. Nutmeg Oil Physical Characteristics Nutmeg Oil of North Sulawesi Mainland vs Islands

Parameter	Sample					
	Islands			Mainland		
	1	2	3	1	2	3
Color	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow
Aroma	Distinct Mild Nutmeg	Distinct Mild Nutmeg	Distinct Mild Nutmeg	Distinct Mild Nutmeg	Distinct Mild Nutmeg	Distinct Mild Nutmeg
Density	0.880	0.870	0.883	0.863	0.860	0.870
Refraction Indexes	1.480	1.472	1.475	1.471	1.469	1.472
Optical Rotation	+ 26.22	+ 30.27	+ 24.03	+ 31.84	+ 28.48	+ 32.10
Solubility in Alcohol	1:6 Soluble	1:6 Soluble	1:6 Soluble	1:6 Soluble	1:6 Soluble	1:6 Soluble

Islands:	Mainland:
1. Sangihe	1.Minahasa Utara
2. Talaud	2. Minahasa Tenggara
3. Siau	3. Minahasa Induk

Tabel 3. Nutmeg Oil Physical Characteristics Nutmeg Oil

Parameter	Banda Nutmeg*	Papua Nutmeg**	SNI-2388-2006
Color	Bening	kuning muda	bening-kuning muda
Density (20°/20°C)	0.945	0.906-0.912	0.885-0.907
Refractive Indexes (20°C)	1.47	1.484 – 1.489	1.475 – 1.485
Optical Rotation (20°C)	+ 17.9	(+12.3) – (+18.2)	(+6) – (+18)
Solubility in Alcohol	1:4 Soluble	1:1 – 1:3 Soluble	1:1 – 1:3 Soluble

The densities of the oil are mainly due to the collection of the heavy molecules that becomes the constituents of the oil. The major component of nutmeg oil consists of oxygenated monoterpene (terpene-o) ($C_{10}H_{16}O$) or other heavy fractions, while on the other hand if an oil consists of more hydrocarbon monoterpene (terpene) ($C_{10}H_{16}$) or lighter molecular weighted compounds then the density will be much smaller [27]. Oxygenated monoterpene has a molecular mass and boiling point higher than that of hydrocarbon monoterpene thus the total molecular mass will be higher. The amount of terpineol and safrole molecules in nutmeg oils originating from Talaud and North Minahasa are like that of Papua, and higher than that of Banda Island, therefore the density of nutmeg oil of Talaud, North Minahasa and Papua are higher than that of from Banda. Molecular weight of terpineol and safrole are greater than that of α-pinene, β-pinene, sabinene, carene, and terpinene, that represents as the main component of nutmeg oil of Banda [28].

The refractive indexes are a comparison of the speed of light in the air and in a material at a certain temperature [22]. The results this research indicated in terms of refractive indexes are 1.4909 for the oil originating from mace of nutmegs of Talaud and 1.4934 of North Minahasa, while from the seeds are 1.4807 from Talaud and 1.4834 for North Minahasa. The refraction index of the oil of maces from Banda and Papua are close to the standard by SNI (1.475-1.485).

The refraction indexes of the oil are mainly influenced by compounds that build the major structures. The higher the number of carbon chain and double bonds the higher the refractive index will be [25]. Myristicin is considered to be a component of the nutmeg oil that has the tendency to raise the refractive indexes as the concentration of myristicin rises. The refractive index of oil from the mace of the nutmeg of Papua, North Sulawesi, and Banda are due to the presences of high amounts of myristicin, susafrole and terpineol. These compounds are known to have long molecular chains and to have more hydroxyl groups, meanwhile, the more long chained components like sesquiterpene or components with oxygen groups that are destilled along, the presences of medium sized components will also be more and thus it will be more difficult to refract light that will cause a higher refractive index [22, 29].

The optical rotation of the nutmeg oil of the mace of Talaud were + 9.80° while North Minahasa was + 6.90°. The optical rotation of the seeds of Talaud nutmeg oil was +22.30° and North Minahasa was + 20.73°. In accordance to the standards by SNI are (+6°) – (+18°), therefore the oils of both seed and mace of nutmegs from Talaud and North Minahasa within standards.

The optical rotations are a response of a single wave of light traveling through the molecular structures. The oil of mace of Talaud and North Minahasa as is with the oil of nutmegs of Papua contains oxygenate hydrocarbon (safrole and terpineol) that gives a smaller positive compared to oil of Banda nutmegs, but would have an even smaller positive response compared to nutmeg oil of North Sulawesi including from Talaud and North Minahasa in this research that originated from the seeds. Banda oil apparently contains higher concentration of aromatic hydrocarbons

(α-pinene, β-pinene, sabinene, carene, and terpinene) and thus would give a more positive rotational response. This would mainly be due to the conditions of fixed oil contents and polymerization results of the trimyristin, where trimyristin contains longer carbon chains and tends to be more assymetric. This nature correlates to the carbon symmetric carbon structure of the oxygenated hydrocarbons and the assymetric carbon structure of the aromatic hydrocarbons [30]. In which positive optical rotation would exhibit a spin of the polarization field to the right while on the other hand a negative rotation are to the left [31].

Solubility in ethanol would a physical trait that would correspond with the polarity and purity of the essential oil. An essential oil that contains more polar components would be more soluble in polar solvents [27]. All our findings concluded that the nutmeg oil of Talaud and North Minahasa, both from the seed and mace all are soluble in 90% ethanol with a ratio of 1:1, this is in accordance also with the SNI standard of solubility in the range of 1:1 – 1:3.

The perfect solubility in ethanol indicates that the oils had identical polarity with ethanol, thus also indicates the ability of the samples to completely be dissolved in ethanol. The solubility in ethanol is much influenced by the polarity of the components contained within the sample, the more of the larger components with similar polarity with ethanol the quicker the samples would dissolve in ethanol. While if otherwise indicated the more concentration of ethanol required to dissolve the nutmeg oil then the more difficult it would be for the oil to be fully soluble in ethanol [31].

Solubility of an essential oil in ethanol would be in conjunction with the chemical components in the oil itself [12]. Compounds such as oxygenated terpene, α-terpineol and terpinen-4-ol are mainly found within the nutmeg oil of the flesh. Oxygenated terpen containing oil would be more soluble in ethanol then terpen containing oils, thus the higher the terpen concentration the lower the solubility because un-oxygenated terpen are non-polar compounds with no functional groups [22].

Science advancements in this field have moved to a more concentrated focus of the bio activity of the components of nutmeg, such as the antioxidant capacities and the antimicrobial activities of the macelignans, a bioactive compound that was isolated and studied on its inhibitory activities against vegetative cells of Bacillus cereus [32]. Further studies on nutmeg's macelignans and its future applications in the industries might as well be the future of advancements of the nutmeg and also might endorse a new horizon on the economic value of nutmeg.

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Conclusion

The North Sulawesi nutmegs in the findings of this research remained consistent with previous researches, while also remained within standard with the standards set by the National Indonesian Standards. The oil of the mace from nutmeg from Talaud had a lighter appearance in color compared to that from North Minahasa, while the density of oil extracted from North Minahasa was more densed compared to Talaud in both seed and mace (0.923 and 0.938 respectively at 25°C). The reflective index of nutmeg oil from North Minahasa was slightly higher than of Talaud both from seed and mace (1.4834 and 1.493 at 25°C), while the optical rotations of oil extracted from the mace were between +6.90° to +9.80° and from the seed were +20.73° to +22.30°.9. Although in terms of myristicin content the North Sulawesi nutmegs indicated higher concentrations of myristicin compared to nutmeg oil from neighbouring islands of Banda and Papua, therefore the North Sulawesi nutmegs are prone to exhibit stronger fragrant aroma distinctive of the nutmeg, thus provides a certain economic aspect of value that may be exploited by the farmers of North Sulawesi other than just selling nutmeg seeds in the local markets. Although the myristicin is still the center of the economic value of nutmeg, especially in the European markets, it is not a major scientific interest in the bio functional component community.

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